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(71) Applicant: BRISTOL-MYERS SQUIBB COMPANY [US/US]; Lawrenceville-Princeton Road, P.O. Box 4000, Princeton, NJ 08543-4000 (US).

(72) Inventors: POSMANTUR, Raud, M.; 10 Rustic Road, Branford, CT 06405 (US). PARVATHENANI, Lav, Kumar; Apartment #C206, 300 Cold Spring Road, Rocky Hill, CT 06067 (US).

(74) Agent: MAKUJINA, Shah, R.; Bristol-Myers Squibb Company, Lawrenceville-Princeton Road, P.O. Box 4000, Princeton, NJ 08543-4000 (US).

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(54) Title: METHOD OF MODULATING MICROGLIAL ACTIVATION FOR THE TREATMENT OF ACUTE AND CHRONIC NEURODEGENERATIVE DISORDERS

(57) Abstract: The present invention provides methods of modulating or inhibiting microglia activation comprising the administration of a compound capable of inhibiting 5-LOX, FLAP, attenuating degradation of IκBα or inhibiting nuclear translocation of the NF-κB active complex for the treatment of Alzheimer's disease, brain ischemia, traumatic brain injury, Parkinson's Disease, Multiple Sclerosis, ALS, subarachnoid hemorrhage or other disorders associated with excessive production of inflammatory mediators in the brain.

METHOD OF MODULATING MICROGLIAL ACTIVATION FOR THE TREATMENT OF ACUTE AND CHRONIC NEURODEGENERATIVE DISORDERS

Field of the Invention

The present invention comprises methods of treating various acute and chronic central nervous system disorders by the administration of FLAP or 5-lipoxygenase inhibitors.

Background of the Invention

Acute and chronic brain injuries can activate resident microglia (resident macrophage-like cells found in the central nervous system) as well as recruit peripheral immune cells to injured brain regions that can exacerbate neuronal damage. Inflammatory processes can induce cell death by (a) the release of proteases and free radicals that induce lipid peroxidation, (b) direct cytotoxic effects or (c) by the phagocytosis of sublethally injured neurons. The attenuation of microglia and peripheral immune cell activation has been correlated with significant neuronal protection in pre-clinical studies of ischemia, traumatic brain injury, spinal cord injury and Alzheimer's disease.

Oxygenase enzymes like cycloxygenase and lipoxygenase can initiate the conversion of arachidonic acid to physiological important metabolites. Cycloxygenase (COX; prostaglandin H2 synthase) is responsible for the formation of prostaglandins and thomboxanes. *See* Versteeg, H. Van, van Bergen en Henegouwen, M.P.V., van Deventer, S.J.W. and Peppelenbosch, M.P. (1999). Cyclooxygenase-dependent signaling: :molecular events and consequences. *FEBS letters* 445: 1-5. Lipoxygenase is responsible for the conversion of arachidonic acid to leukotrienes. Lipoxygenases and Their Metabolites, Plenum Press, NY. Eds. Nigam and Pace-Asciak. (1999). It is hypothesized that prostaglandins are an important step in transducing immune stimuli into CNS responses. There are two known isozymes of COX currently known COX-1 (constituitively expressed) and COX-2 (induction in response to immune stimuli). It has been established that COX-1 and COX-2 are found to be induced and constituitively expressed in peripheral immune cells as well

as brain, with neuronal expression of COX-2 being enhanced following various CNS insults including cerebral ischemia. Tomimoto, H., Akiguchi, I. Watkita, H., Lin, J.X., Budka, H. Cyclooxygenase-2 is also induced in microglia during chronic cerebral ischemia in humans. *Acta Neuropathol* (Berl) 1: 26-30 (2000).

However, little is known about the role of lipoxygenases (or subsequent metabolites including hydroxyeicosatetraenoic acids (HETEs), leukotrienes, lipoxines, and hepoxilins) in regulating brain inflammation or neurodegeneration. There are currently four known human lipoxygenases (5, 8, 12, and 15-lipoxygenase). All isoforms share a common substrate as well as oxygenase activity but differ greatly in sequence. Although, the role of prostaglandins and COX-2 in modulating inflammation and pain has been well elucidated, the importance of LOX enzymes (specifically 5-LOX or 5-lipoxygenase) in brain following injury is still unresolved. Simon, L.S. Role and regulation of cyclooxygenase-2 during inflammation *American Journal of Medicine* 106: 37S-42S (1999).

Summary of the Invention

Thus, according to a first embodiment of a first aspect of the present invention is provided a method of modulating or inhibiting microglia activation comprising the administration to a human in need thereof a compound capable of inhibiting 5-LOX.

According to another embodiment of the first aspect of the present invention is provided a method of modulating or inhibiting microglia activation comprising the administration to a human in need thereof a compound capable of selectively inhibiting 5-LOX over COX-2.

According to another embodiment of the first aspect of the present invention is provided a method of modulating or inhibiting microglia activation comprising the administration to a human in need thereof a compound capable of inhibiting FLAP.

According to another embodiment of the first aspect of the present invention is provided a method of modulating or inhibiting microglia activation comprising the administration to a human in need thereof para-REV5901 (L-655,238), Bay-x-1005, ML-3000, NDGA or ZILEUTON®.

According to a first embodiment of a second aspect of the present invention is provided a method of inhibiting the release of pro-inflammatory substances from activated microglial cells comprising the administration to a human in need thereof a compound capable of inhibiting 5-LOX.

According to another embodiment of a second aspect of the present invention is provided a method of inhibiting the release of pro-inflammatory substances from activated microglial cells comprising the administration to a human in need thereof a compound capable of selectively inhibiting 5-LOX over COX-2.

According to another embodiment of a second aspect of the present invention is provided a method of inhibiting the release of pro-inflammatory substances from activated microglial cells comprising the administration to a human in need thereof a compound capable of inhibiting FLAP.

According to another embodiment of a second aspect of the present invention is provided a method of inhibiting the release of pro-inflammatory substances from activated microglial cells comprising the administration to a human in need thereof para-REV5901 (L-655,238), Bay-x-1005, ML-3000, NDGA or ZILEUTON®.

According to a first embodiment of a third aspect of the present invention is provided a method of treating Alzheimer's disease, brain ischemia, traumatic brain injury, Parkinson's Disease, Multiple Sclerosis, ALS, subarachnoid hemorrhage or other disorders associated with excessive production of inflammatory mediators in the brain comprising the administration to a human in need thereof a compound capable of inhibiting 5-LOX.

According to another embodiment of a third aspect of the present invention is provided a method of treating Alzheimer's disease, brain ischemia, traumatic brain injury, Parkinson's Disease, Multiple Sclerosis, ALS, subarachnoid hemorrhage or other disorders associated with excessive production of inflammatory mediators in the brain comprising the administration to a human in need thereof a compound capable of 5-LOX over COX-2.

According to another embodiment of a third aspect of the present invention is provided a method of treating Alzheimer's disease, brain ischemia, traumatic brain

injury, Parkinson's Disease, Multiple Sclerosis, ALS, subarachnoid hemorrhage or other disorders associated with excessive production of inflammatory mediators in the brain comprising the administration to a human in need thereof a compound capable of inhibiting FLAP.

According to another embodiment of a third aspect of the present invention is provided a method of treating Alzheimer's disease, brain ischemia, traumatic brain injury, Parkinson's Disease, Multiple Sclerosis, ALS, subarachnoid hemorrhage or other disorders associated with excessive production of inflammatory mediators in the brain comprising the administration to a human in need thereof para-REV5901 (L-655,238), Bay-x-1005, ML-3000, NDGA or ZILEUTON®.

According to a first embodiment of a fourth aspect of the present invention is provided a method of attenuating degradation of IκBα comprising the administration to a human in need thereof a compound capable of inhibiting 5-LOX.

According to another embodiment of a fourth aspect of the present invention is provided a method of attenuating degradation of IκBα comprising the administration to a human in need thereof a compound capable of selectively inhibiting 5-LOX over COX-2.

According to another embodiment of a fourth aspect of the present invention is provided a method of attenuating degradation of IκBα comprising the administration to a human in need thereof a compound capable of inhibiting FLAP.

According to another embodiment of a fourth aspect of the present invention is provided a method of attenuating degradation of IκBα comprising the administration to a human in need thereof para-REV5901 (L-655,238), Bay-x-1005, ML-3000, NDGA or ZILEUTON®.

According to a first embodiment of a fifth aspect of the present invention is provided a method of inhibiting nuclear translocation of the NF-κB active complex comprising the administration to a human in need thereof a compound capable of inhibiting 5-LOX.

According to another embodiment of a fifth aspect of the present invention is provided a method of inhibiting nuclear translocation of the NF-κB active complex comprising the administration to a human in need thereof a compound capable of selectively inhibiting 5-LOX over COX-2.

According to another embodiment of a fifth aspect of the present invention is provided a method of inhibiting nuclear translocation of the NF-κB active complex comprising the administration to a human in need thereof a compound capable of inhibiting FLAP.

According to another embodiment of a fifth aspect of the present invention is provided a method of inhibiting nuclear translocation of the NF-κB active complex comprising the administration to a human in need thereof para-REV5901 (L-655,238), Bay-x-1005, ML-3000, NDGA or ZILEUTON®.

Other embodiments of the invention comprise two or more embodiments or elements thereof suitably combined.

Yet other embodiments and aspects of the invention will be apparent according to the description provided below.

Detailed Description of the Invention

As used herein "a compound capable of selectively inhibiting 5-LOX over COX-2" means a compound having 1 to 500-fold or more, particularly 1 to 50-fold and more particularly 1 to 10-fold selectivity for 5-LOX over COX-2 as measured by the ability to attenuate the production of arachidonic acid metabolites from cellular suspensions (derived from blood or cell lines) stimulated with ionophore A23187 as previously described (Salari et al., 1984, Prostaglandins and Leukotrienes, Vol 13: 53-60; Menard et al., 1990, Br. J. Pharmacol 100: 15-20) incorporated by reference herein. For instance, 5-HETE and LTB4 are arachidonic acid metabolites derived from 5-LOX and 12-hydroxy-heptadecatrienoic (HHT) is an arachidonic acid metabolite for cycloxygenase activity. Alternatively, COX-2 can be specifically assessed by the ability to attenuate the production of the arachidonic acid metabolite, PGE2, from cellular suspensions (derived from blood or cell lines) stimulated with

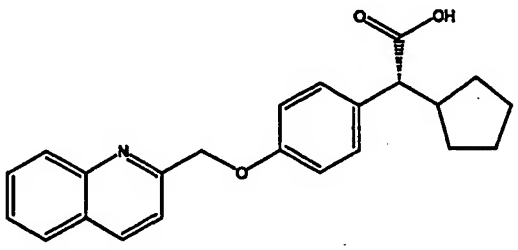
the LPS (Laufer et al., 1999, Inflammation Research, 48: 133-138; Horton et al., 1999; Anal Biochim 271:18-28) .

As used herein "FLAP" means 5-LOX activating protein. Compounds that inhibit FLAP can be measured by the ability to inhibit photoaffinity labeling of a source of purified FLAP (i.e. rat or human). In addition, FLAP inhibitors are confirmed if there is a correlation in the inhibition of leukotriene synthesis *in vitro* cell based assays (i.e. Human PMN leukotriene synthesis) (Evans et al., 1991, Molecular Pharmacology 40:22-27).

As used herein "inflammatory mediators in the brain" includes but is not limited to cytokines, chemokines, prostaglandins and leukotrienes.

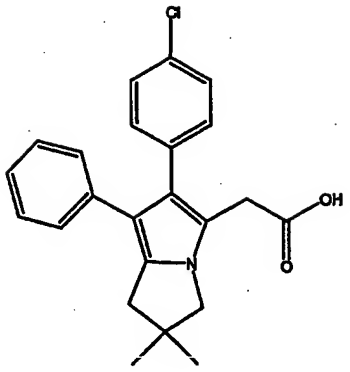
As used herein "pro-inflammatory substances" includes but is not limited to TNF-alpha, nitrite, NO, IL-6, IL-1, 5-HETE, LTB4, LTA4 and other inflammatory substances.

Bay-x-1005 ($C_{23}H_{23}NO_3$) is a selective inhibitor of FLAP. See *Drugs Fut 1995, 20:996* and *Drugs Fut 2000 25(10):1084.*



Bay-x-1005 - $C_{23}H_{23}NO_3$

ML-3000 is an inhibitor of both COX and LOX. See *Drugs Fut 1995 20:1007* and *Drugs Fut 25(10):1093.*



ML-3000 - $C_{23}H_{22}ClNO_2$

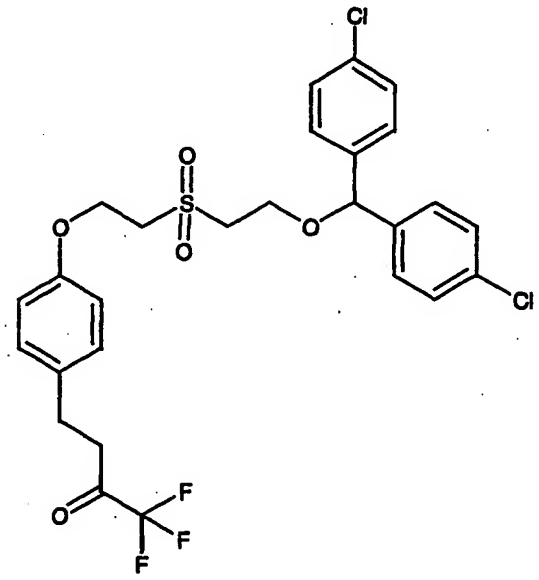
REV5901-para-isomer (L-655,238- IC50= .1uM- 5-LOX) is a selective 5-lipoxygenase activating protein inhibitor (FLAP) with a quinoline structure. It has been reported that FLAP inhibitors with this basic chemical structure interfere with 5-LOX and FLAP protein interactions preventing a required cellular translocation of 5-LOX. Moreover, it has been shown that compounds with the quinoline chemical structure do not affect other routes of arachidonic acid metabolism including known cycloxygenase and other lipoxygenases proteins (Evans et al., 1991, Molecular Pharmacology 40:22-27; Hutchinson, A.W. 1991, Trend in Pharmacological Studies, 12: 68-70).

NDGA is a selective 5-lipoxygenese over cycloxgenase inhibitor (IC50= .2uM- 5-LOX, IC50= 100 uM- COX)-Salari et al, 1984.

We have discovered that indirectly or directly inhibiting 5-lipoxygenase can preferentially attenuate pro-inflammatory cytokine release from activated rat microglia cells in comparison to COX-2 inhibition. While not intending to limit the scope of the invention to any particular mechanism the following description is provided. Cytosolic Ca2+ dependent type IV phospholipase A2 (CPLA2) generates intracellular arachidonic acid (AA). AA is converted to pro-inflammatory prostaglandins, thromboxanes, and leukotrienes by either cycloxygenases (COX) or lipoxygenases (LOX).

Since cytosolic phospholipase A2 (cPLA$_2$) is one of the major enzymes involved in the generation of AA, the effect of lipopolysaccharide (LPS) on cPLA$_2$

was determined. Indirect immunofluorescence with a $cPLA_2$ specific monoclonal antibody revealed that $cPLA_2$ was localized primarily in the cytosol in untreated cells. Upon stimulation with LPS, $cPLA_2$ redistributed to form punctate bodies within 15 minutes and returned to a control immuno-staining pattern by 60 minutes (the transient redistribution of cPLA2 to punctate bodies is an intracellular event associated with higher activity). The activity of $cPLA_2$ can also be enhanced by phosphorylation (Lin et al., 1993). Phosphorylated $cPLA_2$ can be distinguished from unphosphorylated $cPLA_2$ by migration on SDS-PAGE. Immunoblotting revealed that $cPLA_2$ in control cells was predominately unphosphorylated. Following LPS challenge $cPLA_2$ shifted to a phosphorylated form between 10-20 minutes post-challenge. Importantly, CPLA2 inhibitors, *i.e.,* ATFMK (arachidonyltrifluoromethyl ketone) and BMS 229724 have shown significant dose-dependent inhibition of TNF-alpha and nitrite release in LPS activated microglia. The redistribution and phosphorylation of $cPLA_2$ as well as, the attenuation of TNF-alpha and nitrite by cPLA2 inhibitors provide several lines of evidence for the activation of $cPLA_2$ in LPS treated microglia.



BMS-229724 (WO 99/15129)

COX-2 inhibitors rofecoxib (VIOXX®) and celecoxib (CELEBREX®) had no significant effect on pro-inflammatory release on activated microglia. Importantly,

para-REV5901 (α-pentyl-4-(3-quinolinylmethyl)benzenemethanol) a 5-LOX activating protein inhibitor and NDGA (nordihdroguaiaretic acid) a 5-LOX inhibitor, dose dependently inhibited TNF-alpha release and nitrite to near control levels following LPS challenge in microglia cells.

To further validate the role of 5-LOX in pro-inflammatory cytokine release transcriptional regulators of TNF-alpha and NO were examined. Lipoxygenases can activate NFκB mediated transcription via the generation of reactive oxygen intermediates (Lee et al., 1997; Bonizzi et al., 1999). Both the TNFα gene and inducible nitric oxide synthase (iNOS) gene contain NF-κB binding elements in their promoter sequences and activation of NF-κB is crucial for gene transcription (Goldfeld et al., 1990; Drouet et al., 1991; Xie et al., 1994). Hence the effects of inhibiting NF-κB mediated transcription using two distinct inhibitors was assessed with BAY 11-7085 an irreversible inhibitor of IκBα phosphorylation ([IC_{50}-10μM] a biochemical event associated NF-κB activity) and NF-κB SN-50 a cell permeable peptide which inhibits translocation of NF-κB active complex into the nucleus (a required intracellular event associated with NF-κB activity; Lin et al., 1995; Pierce et al., 1997). Both BAY 11-7085 and NF-κB SN-50 inhibited LPS induced TNFα and NO release to control levels.

To further characterize the involvement of NF-κB in microglial signaling, the effect of LPS on the degradation of IκBα and NF-κB (p65) translocation from the cytosol to the nucleus was also determined. It was observed that IκBα was rapidly degraded within 20 minutes following LPS activation and reappeared to control levels by 60 minutes. Consistent with these observations, indirect immunofluorecence with a p65 antibody indicated that in control cells p65 was primarily localized in the cytosol, but after stimulation with LPS p65 rapidly translocated to the nucleus. These results demonstrate that NF-κB mediated transcription can play a role in microglia activation.

To determine whether $cPLA_2$ and 5-LOX regulate TNFα and NO release by influencing NF-κB activation, the effects of $cPLA_2$ and 5-LOX inhibitors on IκBα degradation and nuclear translocation of NF-κB were examined. ATFMK and para-

REV5901 attenuated the degradation of IκBα following LPS stimulation. ATFMK and para-REV5901 also delayed the translocation of NF-κB into the nucleus. These results demonstrate that both $cPLA_2$ and 5-LOX inhibitors attenuate the release of TNFα and NO by delaying IκBα degradation and interfering with NF-κB activation.

These data collectively represent that 5-LOX (via CPLA2, AA, and NF-κB signaling) is a preferential target over COX-2 in modulating or inhibiting microglia activation. Consequently, modulating either 5-LOX alone or in conjunction with COX-2 could have direct effects in enhancing neuronal survival in acute and chronic CNS diseases including Alzheimer's disease, brain ischemia, traumatic brain injury, Parkinson's Disease, Multiple Sclerosis, ALS, and subarachnoid hemorrhage.

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containing a cell membrane-permeable motif and nuclear localization sequence. J Biol Chem 270:14255-14258.

Pierce JW, Schoenleber R, Jesmok G, Best J, Moore SA, Collins T, Gerritsen ME (1997) Novel inhibitors of cytokine-induced IkappaBalpha phosphorylation and endothelial cell adhesion molecule expression show anti-inflammatory effects in vivo. J Biol Chem 272:21096-21103.

Isolation of Microglia from Rat Brains:

Rat microglia were prepared from two day old rat pups. Pup brains were removed and the meninges were gently removed. Once sufficient amount of brains were collected, brains were minced with a blunt scissors (10 times) and transferred to a 15ml conical tube with a pasteur pipette and titurated 25 times. Dissociated cells were then centrifuged at 1000RPM for 10 minutes (RT). The supernatant was removed and 2 mls of fresh media was added. The resultant cell suspension was titurated 10 times. Following titration the cell suspension was plated in a T175 cm^2 culture flasks at a density of 4 brains per flask in 25 mls. MEM media was used for the experiments, supplemented with 10% FBS, 100 i.u.penicillin, 100 i.u.streptomycin and L-Glutamine. Microglia were isolated on day 14 by shaking on an orbital rotation shaker. The purity of the cultures was 98-100% as determined by immunostaining with ED-40 antibody.

Rat Microglia Cell Activation and Drug Exposure

Endotoxin (LPS) at a concentration of a 100ng/ml were used for activation of rat microglia cells. This concentration had previously shown to be effective in inducing TNF-alpha and Nitrite release. All assays were performed in 48 well plates (Becton Dickinson) at ~2 $X10^5$ cells or 0.5 $X10^5$ per 1 ml per well in 10% MEM media. Microglia cells were pre-incubated 1hr prior to LPS challenge with either vehicle (0.1%DMSO) or test compound in DMEM containing 10%FBS (microglia) or RPMI containing 10%FBS (THP-1 monocytes). Supernatants from LPS activated rat microglia were collected at 24 hrs post-LPS challenge.

TNF-alpha ELISA

Collected supernatants were assayed for TNF-alpha using a Pharmingen OPtEIA Rat (microglia).

Nitrite Assay

Nitrite assay was performed in a 96 well plate using a Modified Griess Reagent (Sigma). In brief, a 100ul of Modified Griess Reagent was added to a 100 ul of collected supernatant. Samples were read at a wavelength of 540nM. All values were calculated against a $NaNO_2$ standard curve.

Immunofluorescence

Cells were washed once with PBS, fixed and permeabilized with ice cold methanol (100%) for 5 minutes and washed 3X in PBS for 10 min. The cover slips were blocked for 1 hour in 10% serum/PBS (serum derived from animal in which secondary antibody was generated), incubated for 2-3 hours in primary antibody solution (1:50 dilution in 1.5% serum/PBS) and washed 3X in PBS for 10 min. Secondary antibody linked to fluorescein was applied for one hour (1:100 dilution in 1.5% serum/PBS) and washed 3X in PBS for 10 min. If the nucleus was stained, the cells were incubated for 15 minutes with DAPI (1:10000) at 37°C and washed. The coverslips were then mounted onto glass slides using mounting media and viewed under a fluorescence microscope.

Immunoblotting

Immunoblotting was carried out as described previously (Parvathenani et al., 2000). Briefly 25μg of protein was fractionated on a 4-20% tris-glycine gel (NOVEX, CA) and transferred to PVDF membrane (NOVEX, CA). The membrane was probed with a polyclonal antibody specific for IκBα. To distinguish between the phosphorylated and non-phosphorylated forms of $cPLA_2$, 50μg of protein was run on an 8% tris-glycine gel (Novex, CA) for 4.5 hours at 125V, transferred and probed with a monoclonal antibody specific for $cPLA_2$.

Materials

NDGA (nordihdroguaiaretic acid), para-REV5901 (α-pentyl-4-(3-quinolinylmethyl)benzenemethanol), ATFMK (arachidonyltrifluoromethyl ketone) was obtained from Calbiochem (San Diego, CA). Ibuprofen and LPS was purchased from Sigma (St. Louis, MO). BMS 229724 was synthesized at Bristol-Myers Squibb. NF-κB SN50 and (E)3-((4-t-Butylphenyl)sulfonyl)-2-propenenitrile (BAY-11-7085) were obtained from Biomol (Plymouth Meeting, PA).

Figures:

The data represents mean ± S.D. of triplicate samples of an experiment repeated at least three times. *= Statistically significant ($p<0.05$) in comparison to LPS (positive control).

Figure 1A-E Legend

Microglia were treated with 100ng/ml of LPS for various periods of time following which A-D. $cPLA_2$ distribution was assessed by indirect immunofluorescence (1A) control , (1B) LPS-15 min , (1C) LPS-15 min , (1D) LPS-60 min, (1E) whole cell lysates were prepared and run on SDS-PAGE, transferred and probed with a $cPLA_2$ antibody.

Figure 2A-D Legend

5-lipoxygnease inhibitor (NDGA, 2A) and 5-lipoxygenase activating protein inhibitor (para-REV5901, 2B) significantly inhibited TNF-alpha release, however, COX-2 inhibitors Ibuprofen (2C), Vioxx (2D), and Celebrex (2D) failed to produce any reduction in TNF-alpha release in rat primary microglia cells following LPS activation.

Figure 3A-B Legend

cPLA2 inhibitors ATFMK (3A) and BMS-229724 (3B) significantly inhibited TNF-alpha release in rat primary microglia cells following LPS activation.

Figure 4A-C Legend

cPLA2 inhibitor, ATFMK (4A) and FLAP inhibitor, para-REV5901 (4B) significantly inhibited nitrite release in rat primary microglia cells following LPS activation. However, COX-2 inhibitor, Celebrex (4C) had no effect on nitrite release.

Figure 5A-B Legend

Effects of NF-κB inhibitors, BAY 11-7085 and SN-50 on TNFα and NO release in LPS treated microglia. Microglia were treated with various concentrations of either BAY- or SN-50 for one hour prior to the addition of LPS. Twenty-four hours post LPS challenge the media was assayed for TNFα release by ELISA (5A) and nitrite release by modified Greiss reagent (5B).

Figure 6A-C Legend

Effects of $cPLA_2$ and 5-LOX inhibitors on LPS mediated IκBα degradation. Microglia were treated with 100ng/ml of LPS for various periods of time following which whole cell lysates were prepared and run on SDS-PAGE, transferred and probed with a IκBα antibody as mentioned in immunoblotting. (6A) 100ng/ml LPS alone, (6B) LPS + 10μM ATFMK , (6C) LPS + 50μM L-655,238.

Figure 7 A-D Legend

Effects of $cPLA_2$ and 5-LOX inhibitors on LPS mediated NF-κB translocation. Microglia were treated with 100ng/ml of LPS for various periods of time following which p65 distribution was assessed by indirect immunofluorescence (7A) control,

(7B) LPS-5 min., (7C) LPS + 10μM ATFMK–5 min., (7D) LPS + 50μM L-655,238 –5min. (7E) LPS + NDGA-20μM –5min.

What is claimed is:

1. A method of modulating microglia activation comprising the administration to a human in need thereof a compound capable of inhibiting 5-LOX.

2. A method of modulating microglia activation comprising the administration to a human in need thereof a compound capable of selectively inhibiting 5-LOX over COX-2.

3. A method of modulating microglia activation comprising the administration to a human in need thereof a compound capable of inhibiting FLAP.

4. A method of modulating microglia activation comprising the administration to a human in need thereof para-REV5901 (L-655,238), Bay-x-1005, ML-3000, NDGA or ZILEUTON®.

5. A method of inhibiting the release of pro-inflammatory substances from activated microglial cells comprising the administration to a human in need thereof a compound capable of inhibiting 5-LOX.

6. A method of inhibiting the release of pro-inflammatory substances from activated microglial cells comprising the administration to a human in need thereof a compound capable of selectively inhibiting 5-LOX over COX-2.

7. A method of inhibiting the release of pro-inflammatory substances from activated microglial cells comprising the administration to a human in need thereof a compound capable of inhibiting FLAP.

8. A method of inhibiting the release of pro-inflammatory substances from activated microglial cells comprising the administration to a human in need thereof para-REV5901 (L-655,238), Bay-x-1005, ML-3000, NDGA or ZILEUTON®.

9. A method of treating Alzheimer's disease, brain ischemia, traumatic brain injury, Parkinson's Disease, Multiple Sclerosis, ALS, subarachnoid hemorrhage or other disorders associated with excessive production of inflammatory mediators in the

brain comprising the administration to a human in need thereof a compound capable of inhibiting 5-LOX.

10. A method of treating Alzheimer's disease, brain ischemia, traumatic brain injury, Parkinson's Disease, Multiple Sclerosis, ALS, subarachnoid hemorrhage or other disorders associated with excessive production of inflammatory mediators in the brain comprising the administration to a human in need thereof a compound capable of 5-LOX over COX-2.

11. A method of treating Alzheimer's disease, brain ischemia, traumatic brain injury, Parkinson's Disease, Multiple Sclerosis, ALS, subarachnoid hemorrhage or other disorders associated with excessive production of inflammatory mediators in the brain comprising the administration to a human in need thereof a compound capable of inhibiting FLAP.

12. A method of treating Alzheimer's disease, brain ischemia, traumatic brain injury, Parkinson's Disease, Multiple Sclerosis, ALS, subarachnoid hemorrhage or other disorders associated with excessive production of inflammatory mediators in the brain comprising the administration to a human in need thereof para-REV5901 (L-655,238), Bay-x-1005, ML-3000, NDGA or ZILEUTON®.

13. A method of attenuating degradation of IκBα comprising the administration to a human in need thereof a compound capable of inhibiting 5-LOX.

14. A method of attenuating degradation of IκBα comprising the administration to a human in need thereof a compound capable of selectively inhibiting 5-LOX over COX-2.

15. A method of attenuating degradation of IκBα comprising the administration to a human in need thereof a compound capable of inhibiting FLAP.

16. A method of attenuating degradation of IκBα comprising the administration to a human in need thereof para-REV5901 (L-655,238), Bay-x-1005, ML-3000, NDGA or ZILEUTON®.

17. A method of inhibiting nuclear translocation of the NF-κB active complex comprising the administration to a human in need thereof a compound capable of inhibiting 5-LOX.

18. A method of inhibiting nuclear translocation of the NF-κB active complex comprising the administration to a human in need thereof a compound capable of selectively inhibiting 5-LOX over COX-2.

19. A method of inhibiting nuclear translocation of the NF-κB active complex comprising the administration to a human in need thereof a compound capable of inhibiting FLAP.

20. A method of inhibiting nuclear translocation of the NF-κB active complex comprising the administration to a human in need thereof para-REV5901 (L-655,238), Bay-x-1005, ML-3000, NDGA or ZILEUTON®.

FIGURE 1A-E:

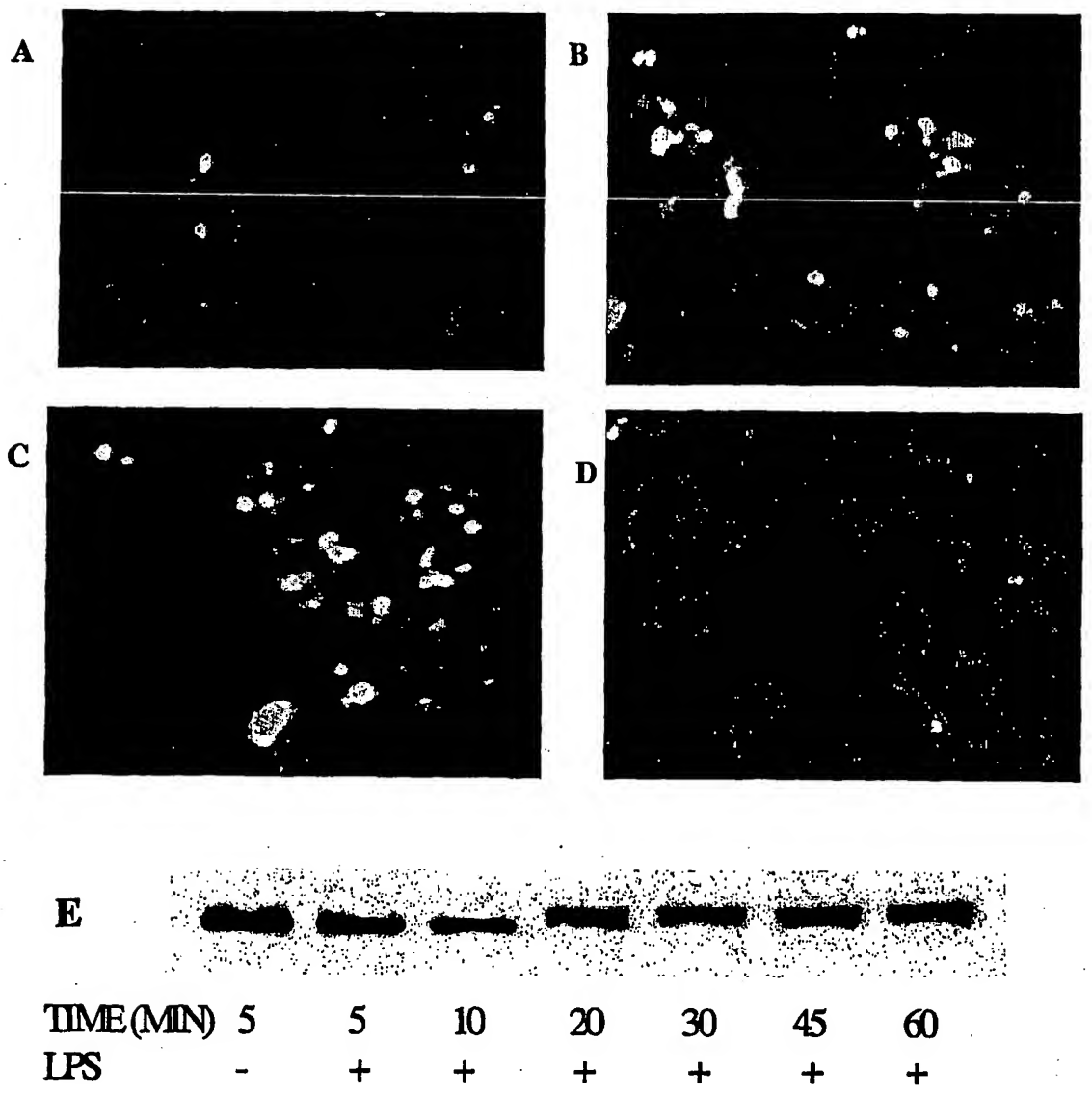


FIGURE 2A:

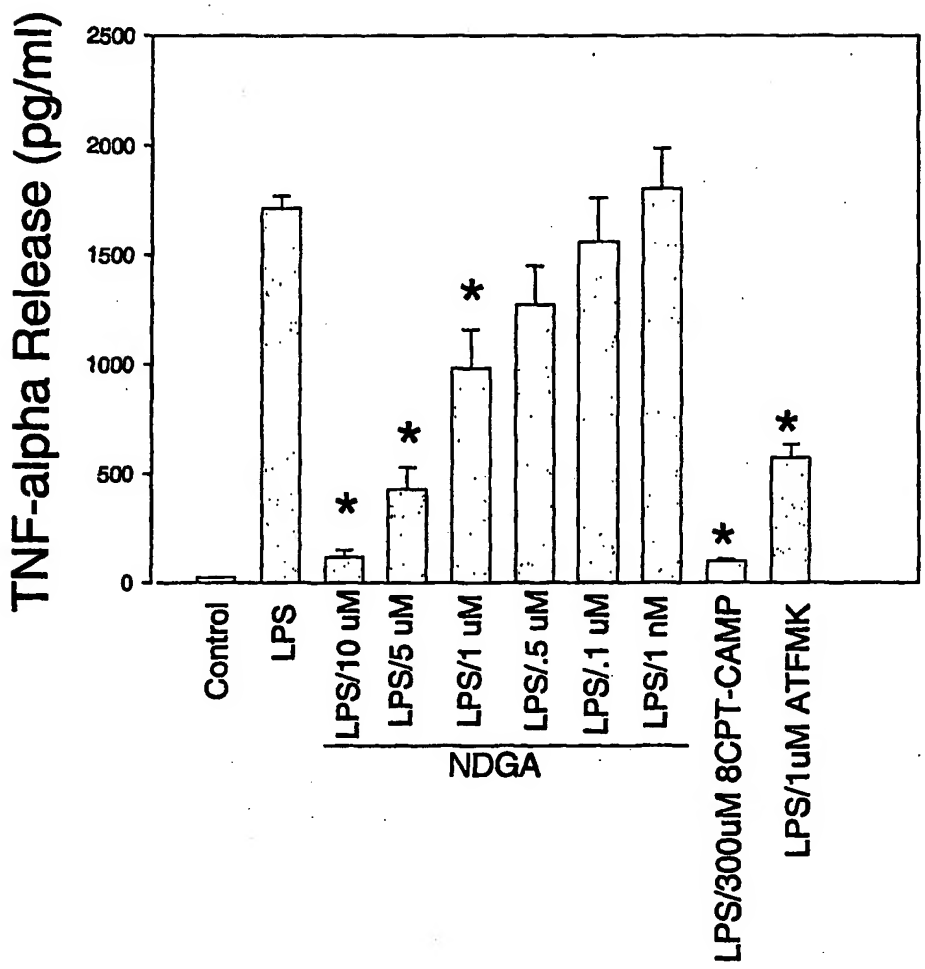

TNF-alpha Release (pg/ml)
2500
2000
1500
1000
500
0
Control
LPS
LPS/10 uM
LPS/5 uM
LPS/1 uM
LPS/.5 uM
LPS/.1 uM
LPS/1 nM
NDGA
LPS/300uM 8CPT-CAMP
LPS/1uM ATFMK
*

FIGURE 2B:

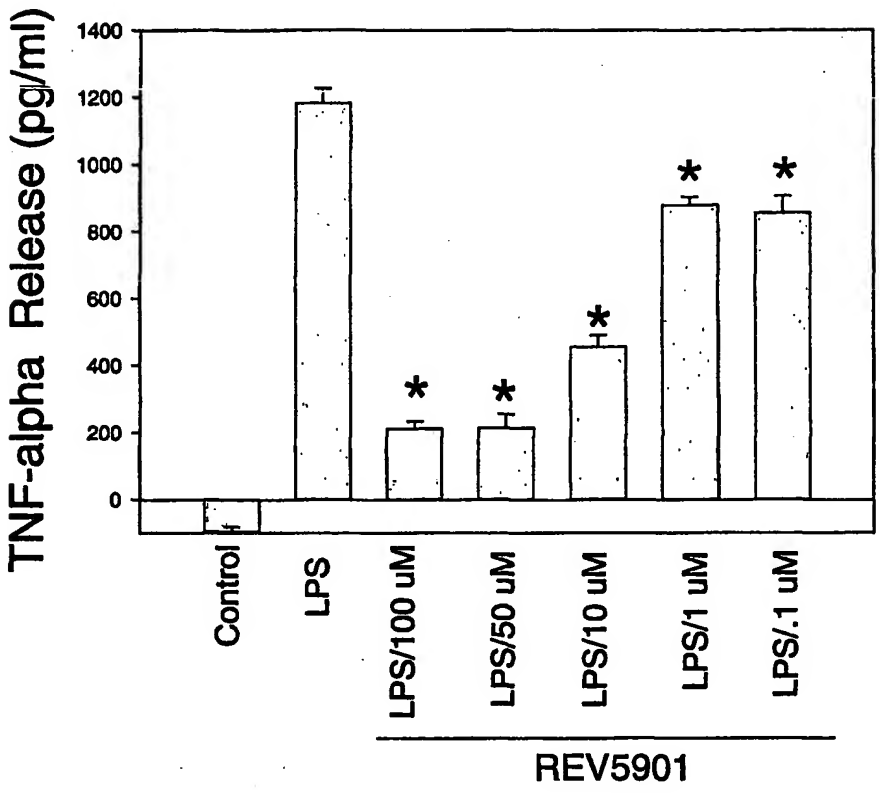

TNF-alpha Release (pg/ml)
1400
1200
1000
800
600
400
200
0
*
*
*
*
*
Control
LPS
LPS/100 uM
LPS/50 uM
LPS/10 uM
LPS/1 uM
LPS/.1 uM
REV5901

FIGURE 2C :

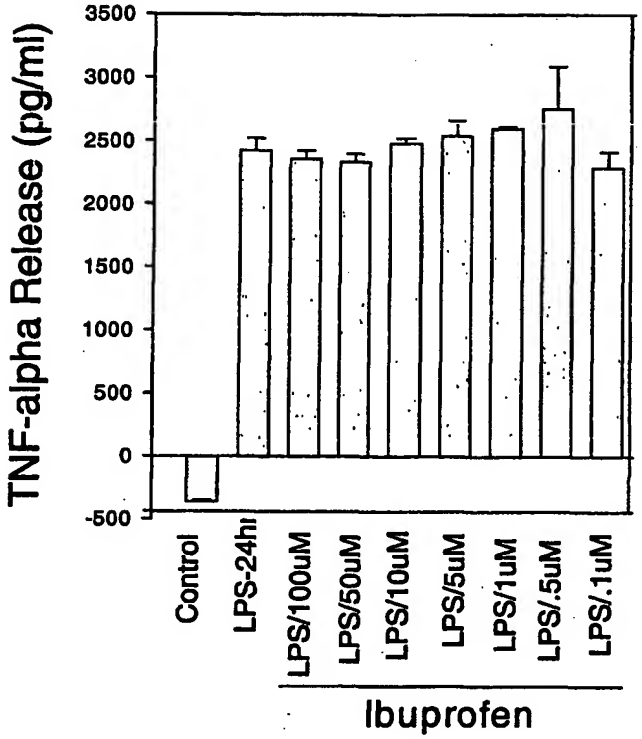

TNF-alpha Release (pg/ml)
3500
3000
2500
2000
1500
1000
500
0
-500
Control
LPS-24hl
LPS/100uM
LPS/50uM
LPS/10uM
LPS/5uM
LPS/1uM
LPS/.5uM
LPS/.1uM
Ibuprofen

FIGURE 2D:

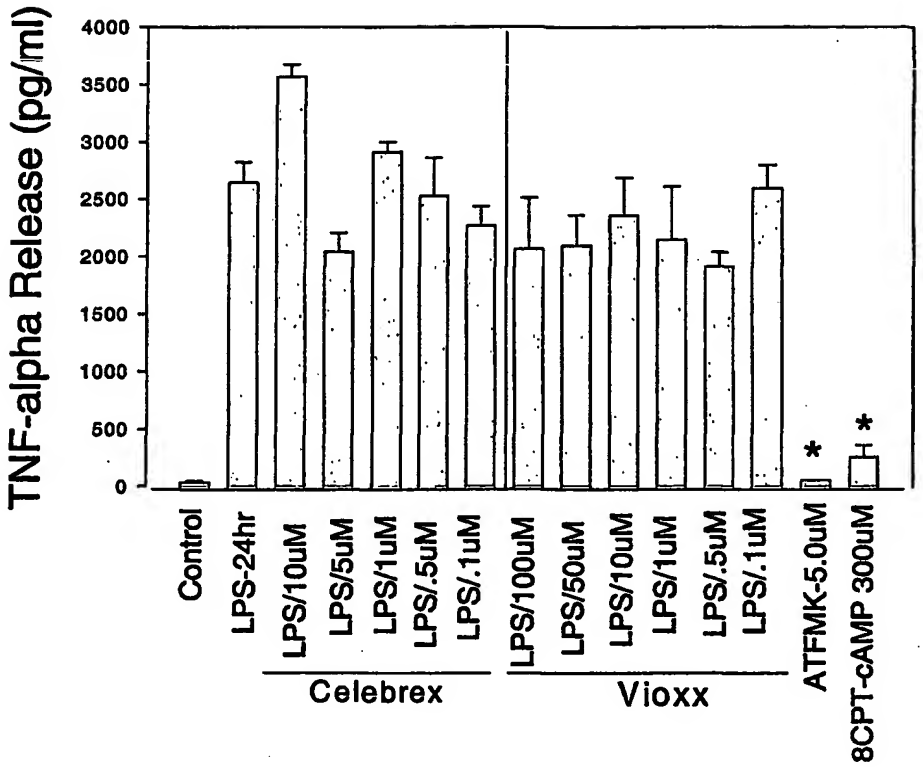

TNF-alpha Release (pg/ml)
4000
3500
3000
2500
2000
1500
1000
500
0
Control
LPS-24hr
LPS/10uM
LPS/5uM
LPS/1uM
LPS/.5uM
LPS/.1uM
LPS/100uM
LPS/50uM
LPS/10uM
LPS/1uM
LPS/.5uM
LPS/.1uM
ATFMK-5.0uM
8CPT-cAMP 300uM
Celebrex
Vioxx
*
*

FIGURE 3A

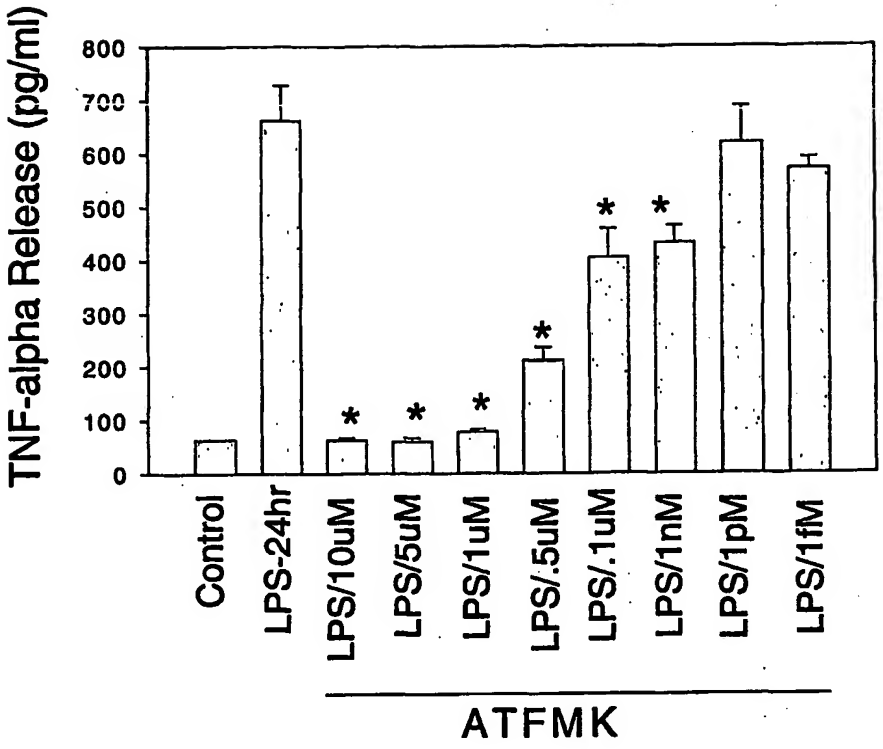

TNF-alpha Release (pg/ml)
800
700
600
500
400
300
200
100
0
Control
LPS-24hr
LPS/10uM
LPS/5uM
LPS/1uM
LPS/.5uM
LPS/.1uM
LPS/1nM
LPS/1pM
LPS/1fM
ATFMK

FIGURE 3B

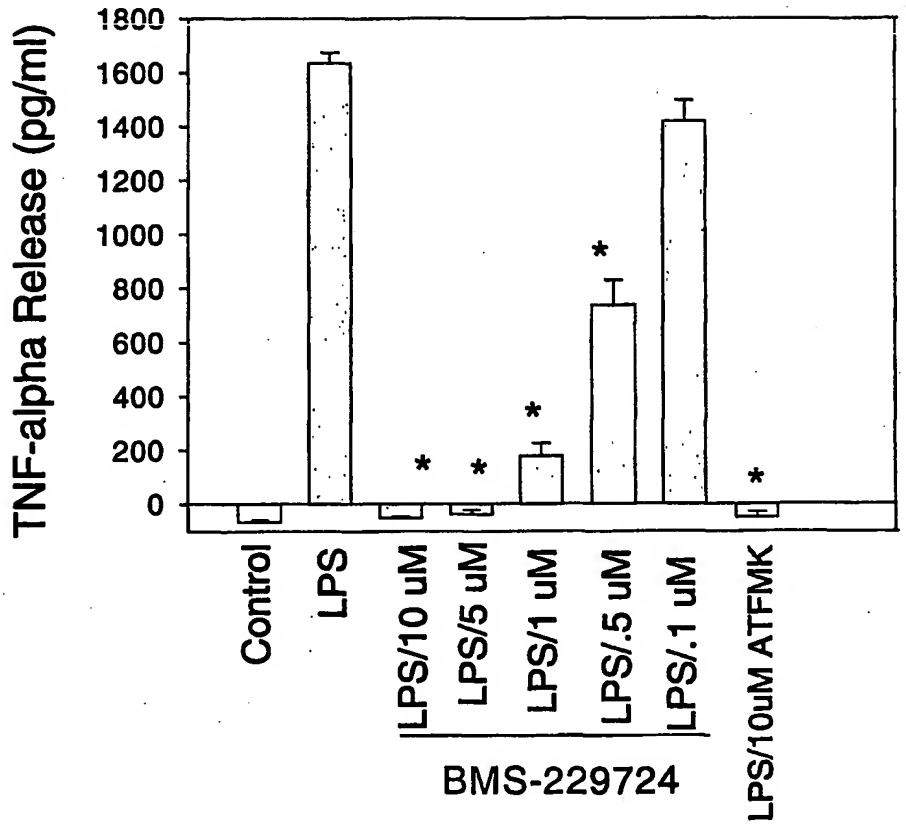

TNF-alpha Release (pg/ml)
1800
1600
1400
1200
1000
800
600
400
200
0
*
*
*
*
*
Control
LPS
LPS/10 uM
LPS/5 uM
LPS/1 uM
LPS/.5 uM
LPS/.1 uM
BMS-229724
LPS/10uM ATFMK

FIGURE 4A

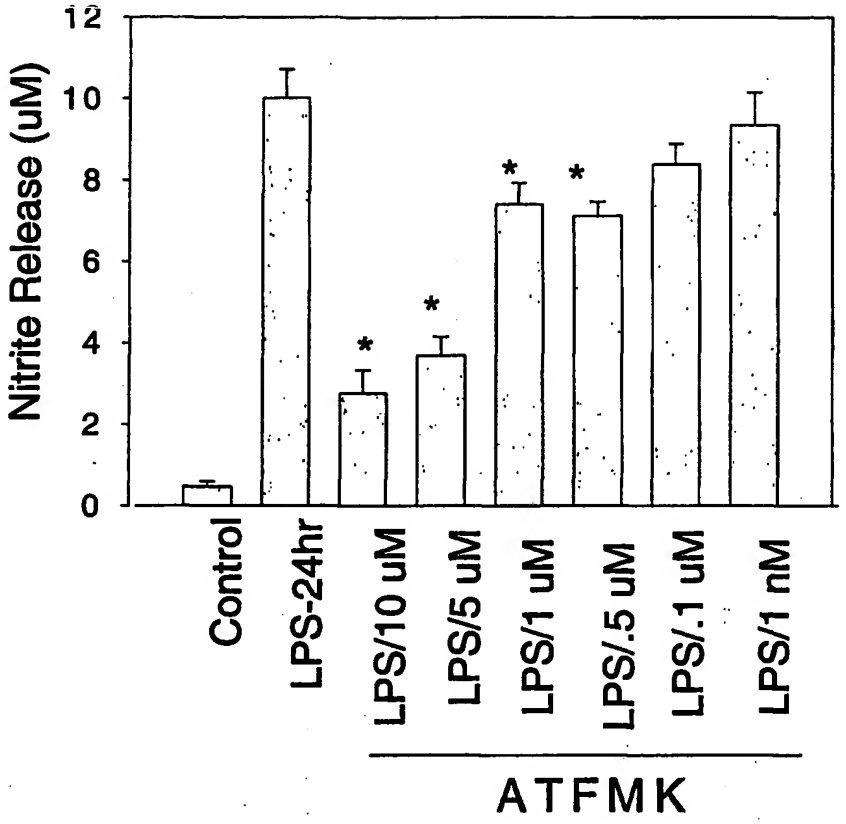

Nitrite Release (uM)
0
2
4
6
8
10
12
*
*
*
*
Control
LPS-24hr
LPS/10 uM
LPS/5 uM
LPS/1 uM
LPS/.5 uM
LPS/.1 uM
LPS/1 nM
ATFMK

FIGURE 4B

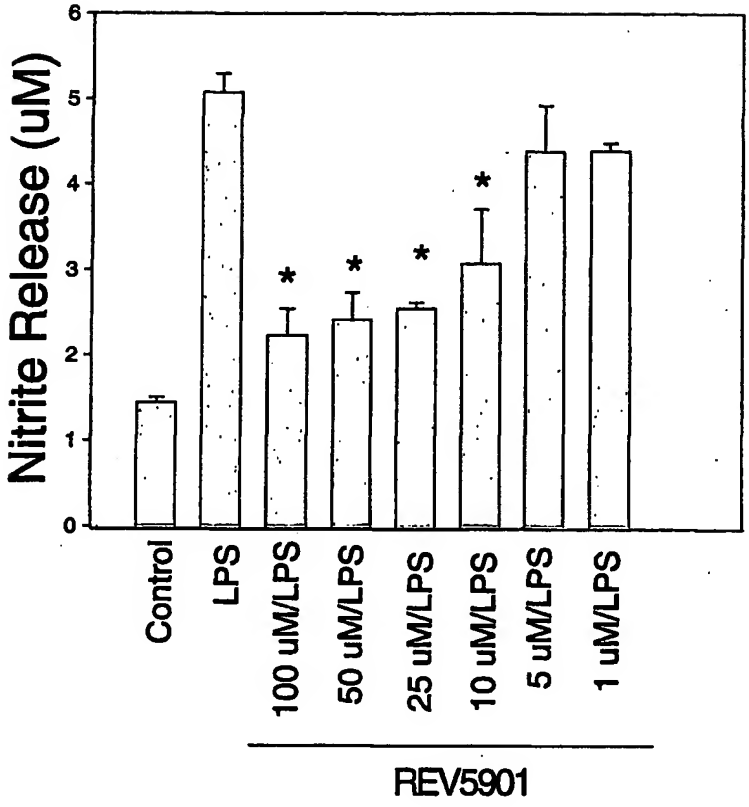

Nitrite Release (uM)
0
1
2
3
4
5
6
*
*
*
*
Control
LPS
100 uM/LPS
50 uM/LPS
25 uM/LPS
10 uM/LPS
5 uM/LPS
1 uM/LPS
REV5901

FIGURE 4C

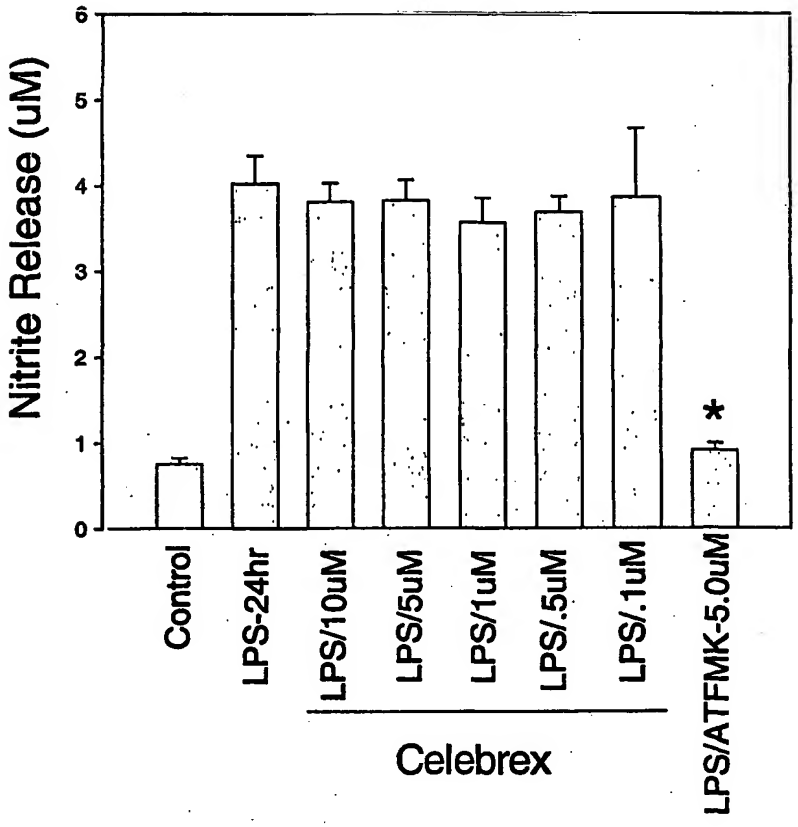

Nitrite Release (uM)
0
1
2
3
4
5
6
*
Control
LPS-24hr
LPS/10uM
LPS/5uM
LPS/1uM
LPS/.5uM
LPS/.1uM
Celebrex
LPS/ATFMK-5.0uM

FIGURE 5A-B

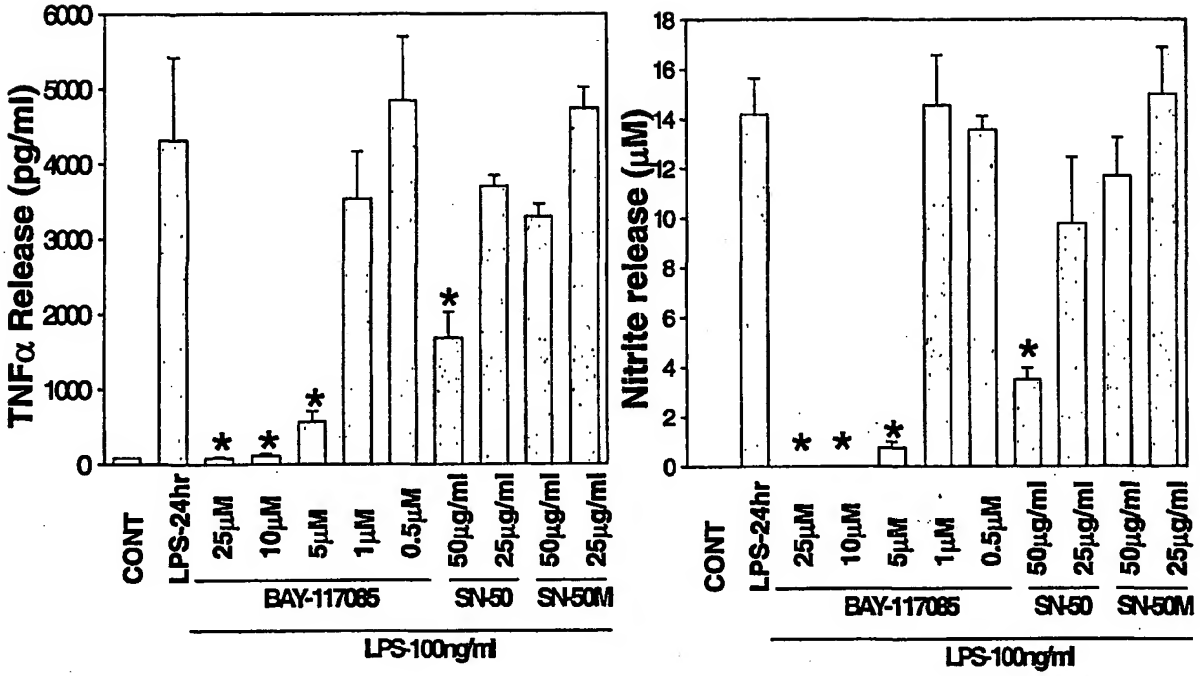

TNFα Release (pg/ml)
6000
5000
4000
3000
2000
1000
0
CONT
LPS-24hr
25μM
10μM
5μM
1μM
0.5μM
50μg/ml
25μg/ml
50μg/ml
25μg/ml
BAY-117085
SN-50
SN-50M
LPS-100ng/ml
Nitrite release (μM)
18
16
14
12
10
8
6
4
2
0
CONT
LPS-24hr
25μM
10μM
5μM
1μM
0.5μM
50μg/ml
25μg/ml
50μg/ml
25μg/ml
BAY-117085
SN-50
SN-50M
LPS-100ng/ml

FIGURE 6A-C

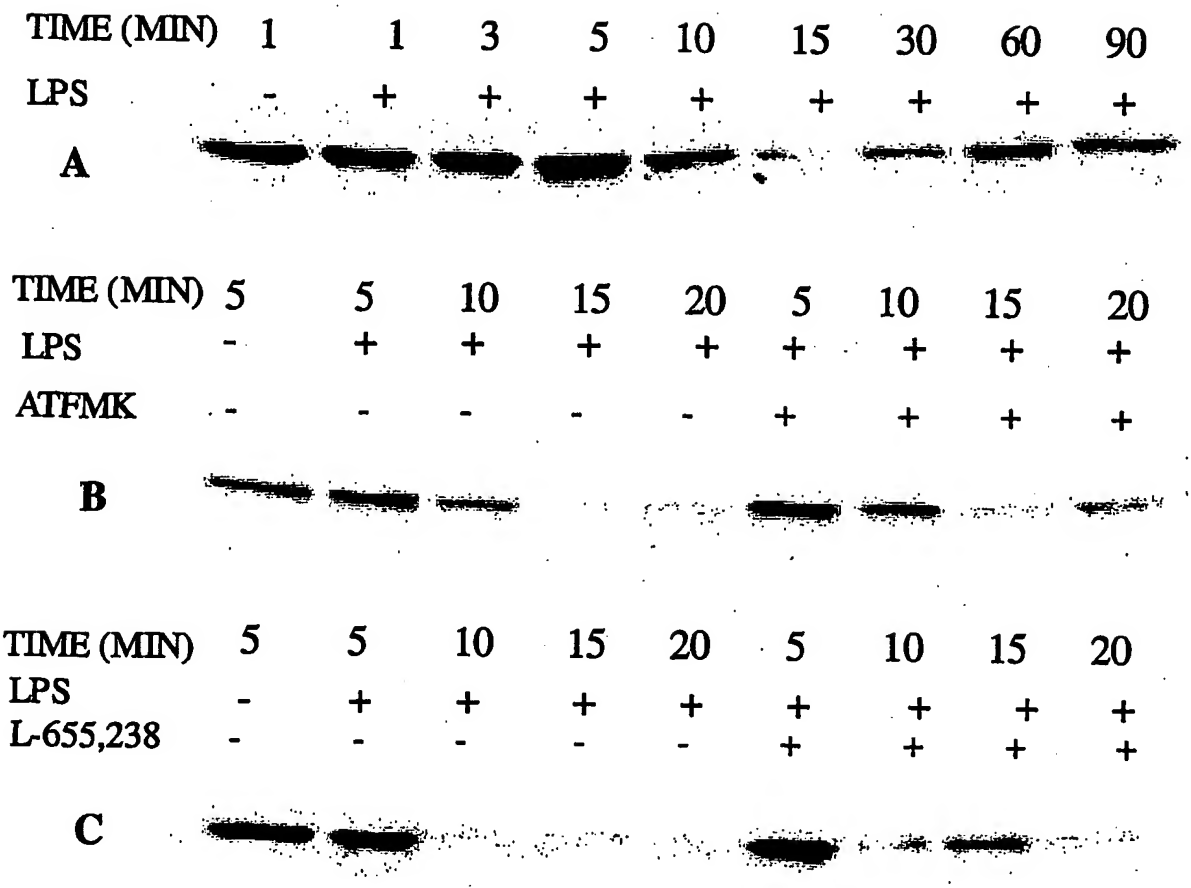

TIME (MIN) 1 1 3 5 10 15 30 60 90
LPS - + + + + + + + +
A
TIME (MIN) 5 5 10 15 20 5 10 15 20
LPS - + + + + + + + +
ATFMK - - - - - + + + +
B
TIME (MIN) 5 5 10 15 20 5 10 15 20
LPS - + + + + + + + +
L-655,238 - - - - - + + + +
C

FIGURE 7A-D

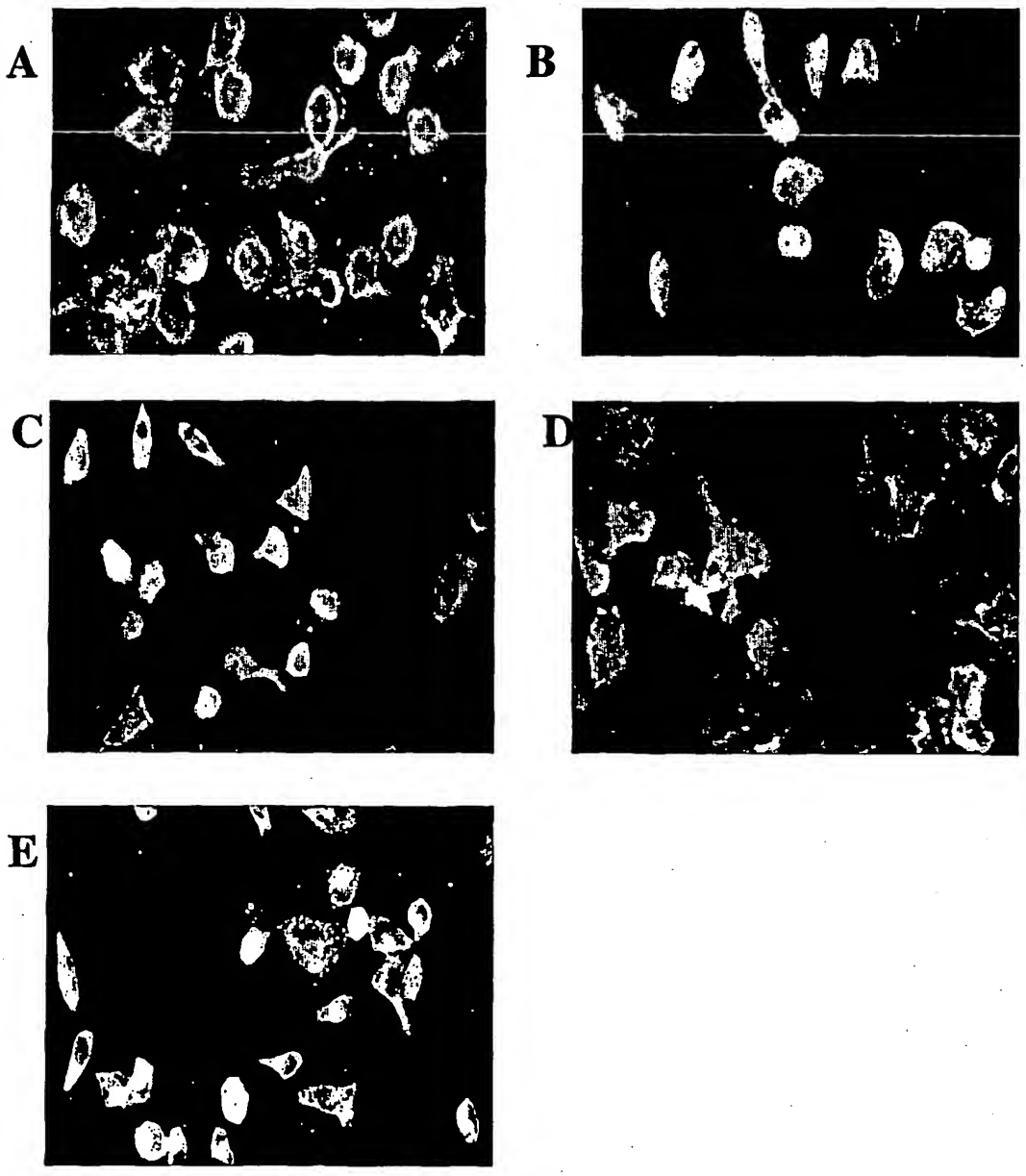
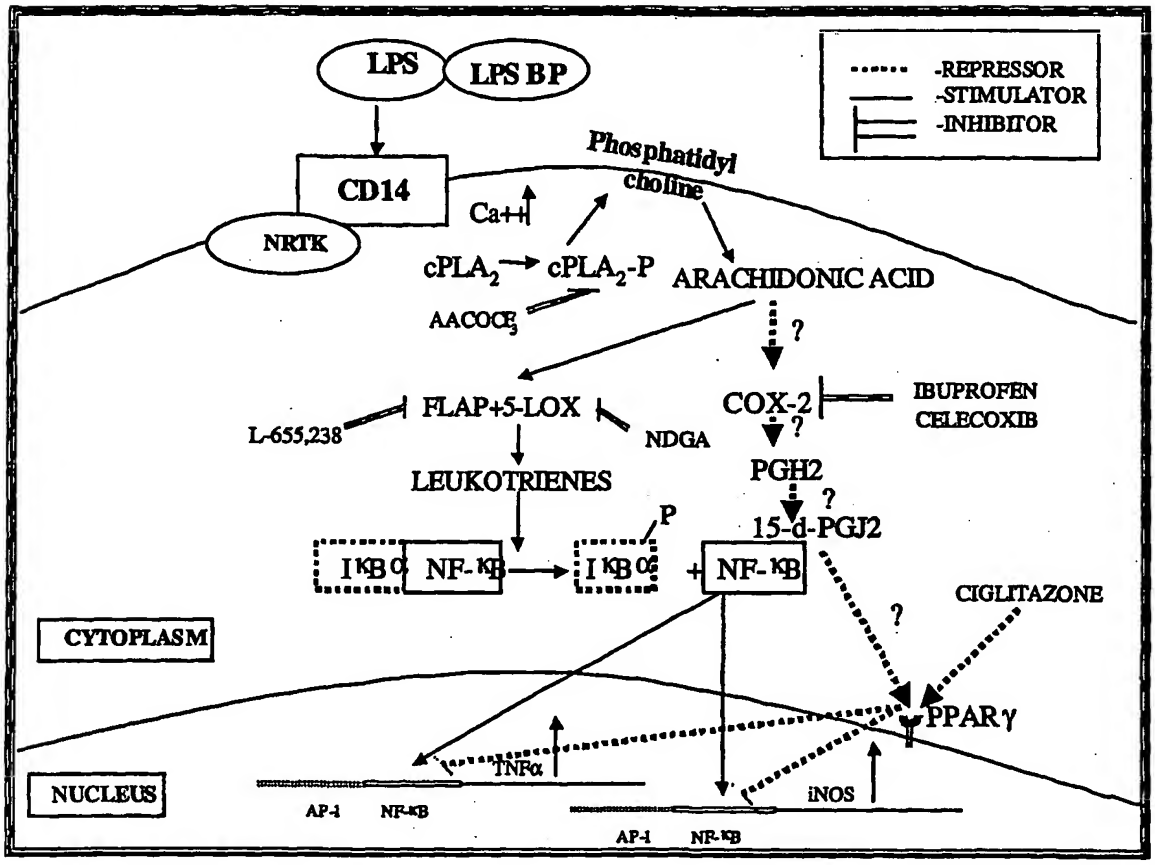


FIGURE 8 Schematic of the Role of 5-LOX in LPS induced microglia activation



5

INTERNATIONAL SEARCH REPORT

Intern al application No.
PCT/US01/21353

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 31/65, 31/38, 31/40, 31/47,
US CL :514/311, 413, 443, 726,
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/311, 413, 443, 726,

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
National Library of Medicine - Medical Subject Headings

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

REGISTRY, CAPLUS, MEDLINE, WPIDS, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Database Medline on STN, (Columbus, OH, USA), No. 1999065, RAMONER, R. ET AL. 'Nordihydroguaiaretic acid blocks secretory and endocytic pathways in human dendritic cells,' abstract, J. Leukocyte Biology, Dec. 1998, 64(6), 747-52.	1-12
A	Database Medline on STN (Columbus, OH, USA), No. 98181109, ABRAHAM, W. ET AL. 'The effects of ML 3000 on antigen-induced responses in sheep,' abstract, Pulmonary Pharmacology Ther., June 1997, 10(3), 167-73.	1-12

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
21 AUGUST 2001	19 NOV 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer REBECCA COOK
Facsimile No. (703) 305-3230	Telephone No. (703) 308-1234

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INTERNATIONAL SEARCH REPORT

Intern ıal application No.
PCT/US01/21353

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Database CAPLUS on STN, (Columbus, OH, USA), LEE, S. ET AL. 'Inhibition of 5-lipoxygenase blocks IL-1.beta.-induced vascular adhesion molecule-1 gene expression in human endothelial cells,' abstract, J. Immunol., 1997, 158(7), 3401-3407.	13-16
A	Database Medline on STN (Columbus, Oh, USA), No. 20178139, JOBIN, C. ET AL. "The I kappa B/NF-kappa B system: a key determinant of mucosalinflammation and protection,' abstract, Am. J. Physiology. Cell Physiology, March 2000, 278(3), C451-62.	13-20